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ALVEOLAR GRADIENT OF PENTANE IN NORMAL HUMAN BREATH

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Previous studies have raised the question of whether pentane is a normal constituent of human breath, since its concentrations in inspired room air and expired breath are often similar. Using a highly sensitive assay for volatile organic compounds, we studied 37 normal subjects in order to determine the alveolar gradient of pentane in their breath (i.e. concentration in alveolar breath minus concentration in the inspired air). The chemical identity of pentane was confirmed by mass spectroscopy. The alveolar gradient of pentane was zero ±0.175 nmol/l in 54.1% of subjects, and distributed in an approximately bell-shaped curve. Determination of the alveolar gradient divided the normal subjects into three groups: the "passive equilibrators" who did not appear to excrete pentane in the breath (the majority), "metabolizers" who actively catabolized inhaled pentane, and "manufacturers" who excreted more pentane than they inhaled.

KEY WORDS: Breath tests, pentane, volatile organic compounds.

INTRODUCTION

Normal human breath contains a large number of volatile organic compounds (VOCs), most of them in very low concentrations (1). In recent years, increasingly refined methods of collection and analysis have demonstrated more than 100 VOCs in the breath, the majority in nanomolar (10^{-9}M) or picomolar (10^{-12}M) concentrations (2–4). Alkanes in the breath, particularly ethane and pentane, have attracted special attention as indicators of lipid peroxidation caused by oxygen free radicals (5). Increased levels of pentane in the breath have been reported in a variety of disorders, including acute myocardial infarction (6), rheumatoid arthritis (7), nutritional deficiency of vitamin E (8), schizophrenia (9, 10) and inflammatory bowel disease (11). However, with improved assay methods has come the realization that several of the VOCs present in the breath can also be detected in room air. It is now apparent that the concentration of pentane in the breath of normal humans may be approximately the same as, or even less than the concentrations observed in the inspired air (10, 12). Such findings prompted Cailleux and Allain (12) to question whether pentane is a normal constituent of human breath.

One of us (MP) has proposed the concept of the alveolar gradient of a breath VOC,

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defined as the concentration of a VOC in the alveolar breath minus its concentration in the inspired air (10, 13). One of the major values of the alveolar gradient is that it indicates the probable source of a VOC, either from the body or from the environmental air. If the alveolar gradient is positive, it indicates that the lungs excreted more of the VOC than they took in, consistent with manufacture of the VOC in the body. Conversely, if the alveolar gradient is negative, it indicates that the lungs excreted less of the VOC than they took in, consistent with ingestion of the VOC from the environmental air and subsequent breakdown in the body.

As part of a clinical study of patients with schizophrenia (10) we had determined the alveolar gradient of pentane in the breath of a group of normal volunteers. We re-examined these data in order to determine if they could throw light on the question of pentane manufacture in normal subjects.

MATERIALS AND METHODS

Human Subjects

These have been described (10). We studied 37 normal volunteers, principally physicians and nurses, with no history of psychiatric illness.

Breath Collection

The method has been described (4). A mobile apparatus was used to collect the VOCs in 20 L alveolar breath by adsorption to an adsorbent cartridge. A sample of background air was also collected.

Assay of Pentane

The method has been described (4). In summary, the VOCs were thermally desorbed from the adsorbent cartridge, separated by gas chromatography, and detected by mass spectroscopy with an ion-trap detector. The identity of pentane in the breath was confirmed by its elution time and mass spectrum, both of which were similar to those observed with pure pentane. The alveolar gradient was determined by subtracting the concentration of pentane in the environmental air from its concentration in alveolar breath.

RESULTS

Determination of Alveolar Gradient

The distribution of the alveolar gradient of pentane is shown in Fig 1. The mean concentration was -0.26 nmol/l (SD $=0.11$).

Confirmation of the Chemical Identity of Pentane

Mass spectroscopy confirmed the identity of pentane in the breath samples. Pentane was distinguished from isoprene which exhibited a different elution time (Figure 2) and a different mass spectrum (Figure 3).

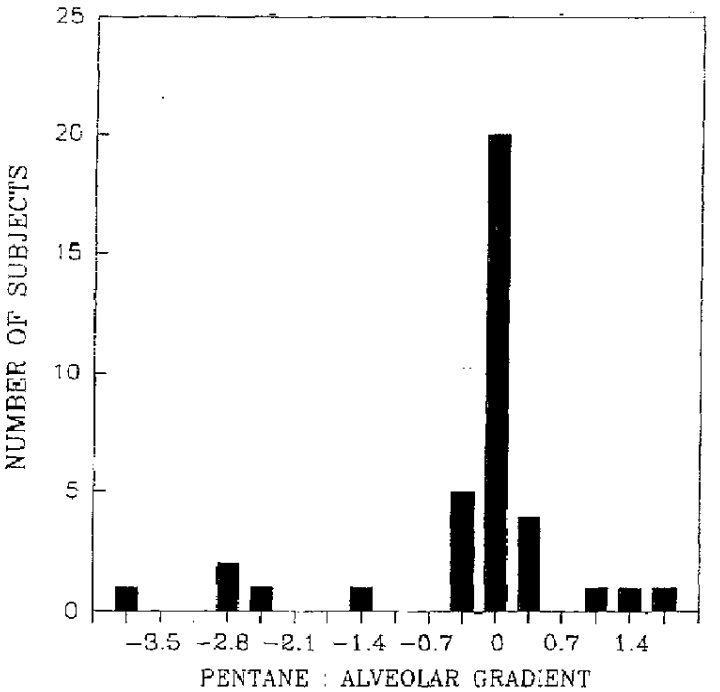

FIGURE 1 Frequency distribution histogram of the alveolar gradient of pentane. Results are shown from assays of the breath of 37 normal subjects. Each bar comprises the number of subjects in the interval shown by the mean ±0.175 nmol/1.

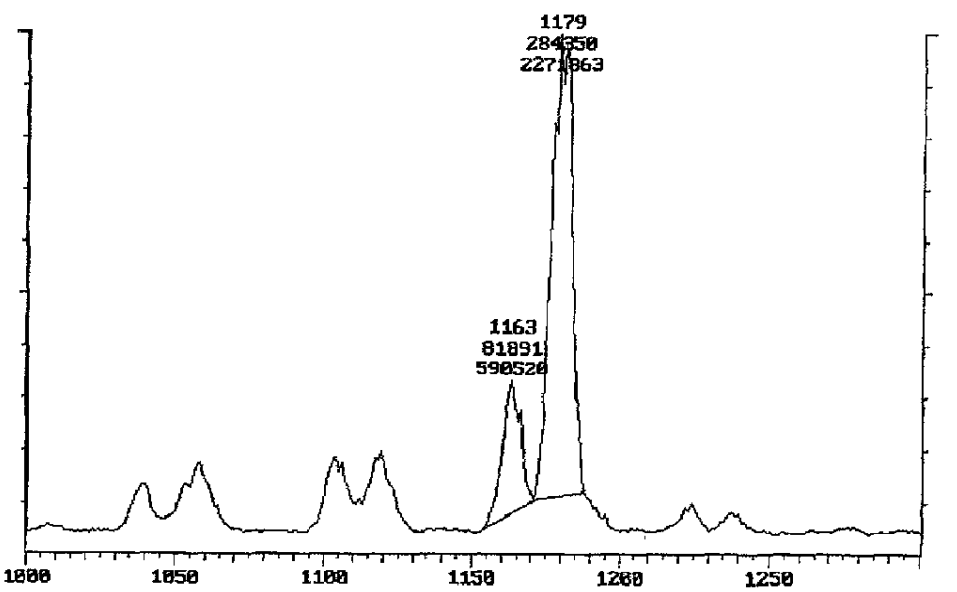

FIGURE 2 Segment of a typical chromatogram of human breath. The *x*-axis indicates scan number (seconds into the run). The numbers superimposed on the two adjacent peaks indicate scan number, peak height, and area under the curve. The peaks are pentane (scan 1163) and isoprene (scan 1179) (see mass spectroscopic confirmation in Figure 3).

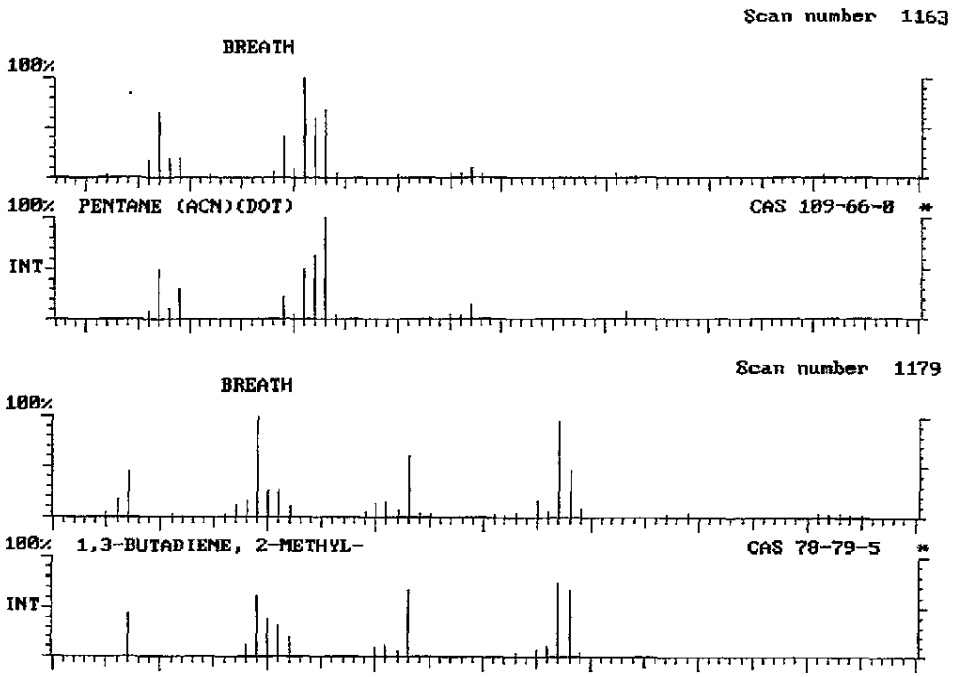

FIGURE 3 Mass spectra of chromatographic peaks. These spectra confirm the identity of pentane (upper panel) and isoprene (2-methyl-1,3-butadiene) (lower panel) in the peaks eluting at scans 1163 and 1179 respectively.

DISCUSSION

The measurement of breath alkanes appears to be a valuable and elegant method for assessing free radical formation and lipid peroxidaton *in-vivo* (14). However, the value of breath pentane measurements has recently been called into question because some assays using gas chromatography have apparently confused pentane with isoprene (15). Pentane and isoprene are both important constituents of the breath which may elute from a column at similar or even identical times. However, this problem did not affect our assay; pentane and isoprene eluted separately and with clearly identifiable mass spectra.

In more than half of the normal subjects, the alveolar gradient of pentane was zero ± 0.175 nmol/l. Values were distributed around zero in an approximately bell-shaped curve. These findings are in general agreement with those of Cailleux and Allain [12]. However, we also observed a small number of subjects whose alveolar gradients of pentane were either markedly negative or positive.

We conclude that most normal subjects did not appear to excrete pentane in their breath. The pentane detected in their alveolar breath most probably resulted from passive equilibration across the alveolar membrane between pentane in the inhaled air and in the venous blood. There are two likely explanations for this finding: either the majority of normal subjects did not manufacture detectable quantities of pentane

in their bodies, or else what pentane they did manufacture may have been rapidly metabolized to other compounds.

In addition, small numbers of the normal subjects exhibited either strongly positive or negative alveolar gradients. Those with negative alveolar gradients had apparently metabolized the pentane inhaled from the air, while those with positive alveolar gradients appeared to be manufacturing pentane in their bodies. The alveolar gradient of pentane therefore divided our normal subjects into three groups: "passive equilibrators" (the majority) and, at the two extremes of the bell-shaped curve, "metabolizers" and "manufacturers" of pentane.

Further research is needed to confirm these findings and to determine their significance. In particular, it would be of interest to learn if pentane "metabolizers" are at lower risk of those diseases mediated by an excess of oxygen free radicals. These findings also raise the possibility that those normal subjects who were "manufacturers" of pentane might have been suffering from the preclinical phase of a disorder associated with increased lipid peroxidation.

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